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Clonal ant societies exhibit fertility-dependent shifts in caste ratios

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Caste differentiation leading to reproductive division of labor is the hallmark of insect societies. Insect colonies typically contain mated queens that reproduce and workers with reduced fertility that undertake the tasks required for colony maintenance and development. Despite the prediction that the proportion of morphological castes should vary to enhance the fitness of colony members in response to environmental conditions, shifts in caste ratios have so far only been reported in a competitive situation. Societies of the ant *Cerapachys biroi* have evolved in an extraordinary way, in that queens and all workers reproduce through obligatory thelytokous parthenogenesis. Because workers of *C. biroi* represent the main reproductive force of the colony, the presence of such unmated queens seems puzzling. Here, we show that societies of *C. biroi* alter caste ratios by considerably increasing the production of queens when larvae are reared by sterile individuals in 2 situations: when senescent colonies are faced with food shortage or when well-fed larvae are reared by callow workers due to persisting plentiful resources. In the absence of these opposite conditions, larvae mostly develop into workers. Additional experiments suggest that these results are consistent with a contact pheromone to which larvae could be exposed when cared for by fertile individuals. In this species in which reproduction mainly relies on young workers with finite fertility, a self-regulated mechanism of caste differentiation could allow the enhancement of colony growth through worker production in fertile conditions or the restoration of colonial fertility through queen production in senescent societies. *Key words:* cannibalism, caste differentiation, *Cerapachys biroi*, fertility signal, polyphenism, thelytokous parthenogenesis. *[Behav Ecol]*

An important transition in the evolution of insects was the shift from a solitary life to societies of organisms exhibiting a division of labor based on specialized castes. This adaptive division of labor is at the basis of the ecological success of insect societies and is achieved either by the nestmates assuming temporary behavioral functions and/or by the evolution of permanently differentiated morphological castes (Oster and Wilson 1978; Hölldobler and Wilson 1990). Even if genetic influences on female caste determination or task specialization have been demonstrated (reviewed in Smith et al. 2008), female eggs are usually totipotent, and a developmental switch during the larval stage controlled by nutritional, social, and other environmental factors results in pronounced physical and physiological specializations among female castes (Wilson 1971; Oster and Wilson 1978; Wheeler 1986; Hölldobler and Wilson 1990; Vargo and Passera 1991; Wheeler 1991; O'Donnell 1998; Karsai and Hunt 2002). In ants, particularly, queens are typically adapted for dispersal, mating, colony founding, and are specialized in egg laying. In contrast, wingless and generally smaller than queens, workers exhibit reduced ovaries and lack a spermatheca in most species. They rarely reproduce and mostly care for the brood, forage, and defend the colony (Wilson 1971; Hölldobler and Wilson 1990). Moreover, in approximately 15% of the ant genera, the worker force can also be differentiated into multiple castes (Oster and Wilson 1978; Hölldobler and Wilson 1990).

Because natural selection acts both at the individual and at the colony level, it has been suggested that the various physical castes exhibited by social insects have evolved to enhance the inclusive fitness of colony members with the prediction that caste ratios reflect the colony's needs and should vary with environmental factors, such as predation, competition, or food availability (Wilson 1971; Oster and Wilson 1978; Herbers 1980; Lumsden 1982; Wilson 1985; Schmid-Hempel 1992, Hasegawa 1997). However, this prediction for adaptive shifts in caste ratios has received little evidence and has so far only been reported in a competitive situation (Passera et al. 1996; Harvey et al. 2000; McGlynn and Owen 2002; Yang et al. 2004).

Far from the social organization, commonly observed in insect societies, colonies of the ant *Cerapachys biroi* have evolved in an extraordinary way, in that there is neither a sexual reproduction (mating) nor a sterile caste. Egg laying is distributed among all nestmates through obligatory thelytokous parthenogenesis (Tsuji and Yamauchi 1995) and without any social hierarchy (Ravary and Jaisson 2004). Reproduction is then divided between 2 discrete morphological castes. On the one hand, workers that lay eggs (1 or 2 eggs/month) and care for the brood in their youth, later cease to reproduce as they become foragers (after 3–4 months on average; Ravary and Jaisson 2004). On the other hand, atypical queens display higher (5 eggs/month on average; Ravary and Jaisson 2004) and lasting laying capacities (at least 1 year; Lecoutey E, personal observation). Apterous (Brown 1975, Morisita et al. 1989) and devoid of spermathecae (Tsuji and Yamauchi 1995), these ergatoid queens represent less than 6% per colony in field colonies (Ravary F, personal communication) and are only involved in reproduction and brood care (Ravary and Jaisson 2004).

Because workers of *C. biroi* reproduce through thelytoky and represent the main reproductive force of the colony (94% of workers laying 1–2 eggs during 3–4 months on average vs. 6% of ergatoid queens laying 5 eggs/month on average; Ravary

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and Jaisson 2004), the presence of such unmated queens seems puzzling. Because the organization of every social insect species has evolved in response to the complex environmental pressures and opportunities unique to its evolutionary history, we hypothesized that queen production in *C. biroi* colonies could be a response to colonial sterility. To determine the role of the queens and how their production is regulated, we therefore monitored colonies' responses to varying internal (fertility) and external (food resources) colonial parameters.

MATERIALS AND METHODS

Study species

As for most Cerapachyinae ants (Hölldobler and Wilson 1990), colonies of *C. biroi* are specialized predators of the brood of myrmicine ants on which they prey through massive raids. In Okinawa (Japan) and Taiwan, colonies of *C. biroi* are characterized by a phasic reproductive cycle composed of 2 alternating phases of activity: During the foraging phase (16.07 ± 2.27 days), old workers explore for prey, whereas a single cohort of larvae develop synchronously, nursed by young workers and queens. Then, during the following statary phase (17.92 ± 0.97days), larvae pupate, all workers stay in the nest, and new eggs are laid by young workers and queens. After the eggs hatch and the young workers emerge, also synchronously, a new foraging phase begins (Ravary and Jaisson 2002, Ravary et al. 2006).

Workers of *C. biroi* therefore separate into 2 behavioral subcastes according to age and thus to reproductive capacities: Old extranidal workers, with no or weak ovarian activity, are specialized in foraging and rarely care for larvae, whereas young intranidal workers remain inside the nest and perform most of the egg laying as well as most of the nursing activities (Ravary and Jaisson 2004). Finally, ergatoid queens differ morphologically from workers by a higher ovariole number (4–6 instead of 2 or 3 for workers), the presence of ocelli, more or less developed vestigial eyes, and an alitrunk with variable degree of fusion of the thoracic tergites (Ravary and Jaisson 2004) (Figure 1). These ergatoid queens are not all identical and present a well coordinated and graded development of morphological characteristics. The deeper the thoracic sutures are, the more developed the vestigial eyes and the ovaries (Ravary and Jaisson 2004, Lecoutey E, personal observation).

Rearing conditions of larvae

Larvae of *C. biroi* were subjected to 8 rearing conditions: They were reared by 4 homogeneous groups (fertile queens, young workers, old workers, and callow workers) in 2 feeding conditions (either well fed or undernourished). Among workers, callow workers are not yet fertile, young workers (1–2 months old) are fully fertile, whereas old workers (more than 4 months old) present no or weak laying capacities. This allows us to test for the potential effects of different parameters known to influence caste differentiation in insect societies (Wilson 1971; Oster and Wilson 1978; Wheeler 1986; Hölldobler and Wilson 1990; Vargo and Passera 1991; Wheeler 1991; O'Donnell 1998; Karsai and Hunt 2002).

Indeed, in many social insects, the queens produce a fertility signal that commits larvae to the worker caste (Wheeler 1986; Hölldobler and Wilson 1990; Vargo and Passera 1991; Keller and Nonacs 1993). However, because young workers of *C. biroi* are also fertile, we predicted that, whatever the food resources, queens and young workers will induce worker development in larvae. We used old workers as a control. However, because we cannot rule out potential behavioral manipulation of larval fate by adults (Bourke and Ratnieks 1999, Hammond et al. 2002) and because old workers do not care for the brood as much as young workers and queens (Ravary and Jaisson 2004), we also used callow workers as a second control (individuals which exhibit nursing activities but are considered unfertile because they have not yet developed ovaries) to avoid any possible misleading conclusions about the respective influence of feeding and nursing. This second control also mimics a highly fertile colony where larvae are almost exclusively reared by callow workers after a previous foraging cycle with plentiful resources.

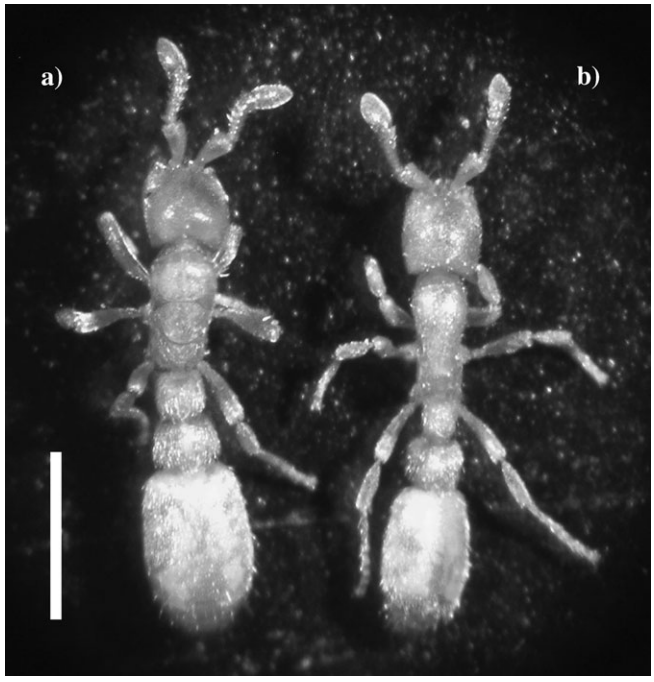

Figure 1
(a) Ergatoid queen and (b) worker of the parthenogenetic ant *Cerapachys biroi*. Scale bar, 1 mm.

In addition, it is well known that the amount of the food given by workers to developing larvae profoundly influences the future adult morphology, including the features critically correlated with fecundity. Well-fed larvae usually differentiate into gyne or soldier, whereas in the absence of sufficient nutrition larvae will develop as workers (Wilson 1971; Oster and Wilson 1978; Wheeler 1986; Hölldobler and Wilson 1990; Wheeler 1991; O'Donnell 1998; Karsai and Hunt 2002). Therefore, we predicted that, in absence of a fertility signal (i.e., in callow and old workers colonies), only well-fed larvae will develop into queens. According to our hypotheses, we expected queen production in only 2 of the 8 experimental conditions: in well-fed colonies of callow workers as well as in well-fed colonies of old workers.

Experimental procedure

Colonies of *C. biroi* were set up in nests consisting of a single rectangular (length: 2 cm × width: 1 cm × height: 0.6 cm) chamber dug in the center of a circular plastic box (8 cm in diameter and 5 cm in height) half filled with a layer of plaster. Nests were covered and closed with a glass plate and a red-colored Plexiglas to allow observations. The plaster was humidified 3 times a week to keep the nests damp. Colonies were kept at 28°C, 70% humidity, and a 12/12 light-dark (LD) photoperiod.

Food and (sub)caste influences
Each of the 32 experimental colonies (8 different conditions performed with experimental colonies originating from 4 wild

colonies collected in Taiwan in 1997, 2000, and 2001) was composed of 28.6 ± 7.2 (mean ± standard deviation [SD]) first instar larvae of the same age and 50 individuals of 1 of the 4 following types: queens (unknown ages and fertile), callow workers (1 day old and sterile), young workers (1–2 months old and fertile), or old workers (more than 4 months old and no or weak laying capacities). Callow workers were isolated at emergence thanks to their light cuticle. Young fertile workers were initially picked up at emergence, but experimental colonies were kept 1 reproductive cycle before starting the experiments. Old workers used for the experiments were only foragers with the darkest cuticle. For each of these 4 types of individuals, 2 colonies were set up with alternate diets: either well fed or undernourished. During the foraging phase of the cycle, colonies were fed 3 times a week with white pupae of *Tetramorium bicarinatum*. Before supplying food, larvae were counted. For every 10 larvae, well-fed colonies received 4 pupae of *T. bicarinatum*, whereas undernourished colonies only got 1. For instance, if a well-fed colony contained 25 larvae, it was supplied with 10 prey items. Because cannibalism regularly occurs in this *myrmecophaegous* species (Lecoutey E, unpublished data), this could be a mean for larvae to selfishly influence their caste development toward queens or for workers to suppress queen-destined larvae (Bourke and Ratnieks 1999; Faustino et al. 2002; Wenseleers and Ratnieks 2004; Wenseleers et al. 2005; Ribeiro et al. 2006; Rüger et al. 2008). For each colony, the proportion of cannibalism was measured with the following ratio: (initial number of larvae − number of prepupae)/initial number of larvae. Indeed, using an indirect measure was necessary because we were unable to separate cannibalism among larvae (self-determination) from cannibalism of larvae by workers (caste regulation) because the same larva can be killed by workers, eaten by larvae, and vice versa. Because old workers tend to forage more than care for the brood (Ravary and Jaisson 2004), nests of all colonies were closed to limit differences in feeding and nursing levels between experimental conditions.

Signal volatility

The results (see below) showed that, in well-fed conditions, colonies of fertile workers strongly prevent queen development in larvae, whereas colonies of callow workers do not. To investigate whether this inhibitory effect was due to a contact or a volatile pheromone, we replicated these 2 experimental conditions in a new experiment with colonies this time set up side by side in a special nest composed of 2 chambers (1 cm × 2 cm each, 1 for each condition), separated by a fine mesh.

Determination of the caste ratio

The potential influence of the rearing caste, food resources, and signal volatility on larval fate was assessed in each colony at the emergence of the new cohort of individuals. Callow individuals were then isolated in a separate nest and dissected 2 weeks later under a stereomicroscope to assign them as workers or queens according to the morphological characteristics of each caste (see above and Figure 1).

Statistical analyses

Food, (sub)caste influences

After arcsine transformation of the proportion of queens produced, the effects of treatments, food, and (sub)castes were compared with an Analysis Of Variance (ANOVA) followed by a post hoc Honestly Significant Difference (HSD) Tukey's test.

Cannibalism

We used an ANOVA after arcsine transformation to compare the effect of food and (sub)castes on cannibalism. Correlations between the proportion of queens produced and the proportion of cannibalism were performed using Pearson's correlation test.

Signal volatility

The proportion of each caste produced in well-fed colonies ($n = 4$) of callow workers separated by a mesh from a well-fed colony of young fertile workers were compared with those obtained in well-fed colonies of callow workers ($n = 4$) using an ANOVA after arcsine transformation. Statistical significance was accepted at $\alpha = 0.05$. All statistical analyses were implemented with StatXact-7 and Statistica 8 softwares.

RESULTS

Of the 8 experimental conditions (Figure 2), numerous queens were reared in undernourished colonies of old workers and well-fed colonies of callow workers (mean ± SD: 79.8% ± 14 and 75.8% ± 10.7, respectively, $n = 4$ colonies for each condition). A moderate proportion of queens was produced in well-fed colonies of old workers (21.2% ± 11.2, $n = 4$), whereas low proportions of queens were reared in the 5 other conditions (mean ± maximum SD: 0–8.1% ± 5.7, $n = 4$ for each condition) (Figure 2).

Consequently, *C. biroi* larvae highly differ in their caste fate according to their rearing conditions (one-way ANOVA, treatment effect: $F_{7,24} = 31.314$; $P < 10^{-5}$). Overall, there was a highly significant influence of the rearing individuals on caste differentiation (one-way ANOVA, (sub)caste effect: $F_{3,28} = 8.473$; $P < 0.001$). Indeed, callow workers and old workers reared globally more queens than young fertile workers and queens (post hoc HSD Tukey's test, all $P < 0.05$). However, although there was no food effect on caste differentiation (one-way ANOVA, food effect: $F_{1,30} = 0.427$; $P = 0.518$), the type of rearing individuals cannot solely explain the results obtained. Indeed, a highly significant interaction existed between feeding conditions and the type of individuals that reared larvae (factorial ANOVA: $F_{3,24} = 33.353$; $P < 10^{-5}$).

In addition, when separated by a single fine mesh from a well-fed colony of young fertile workers, a well-fed colony of callow workers still reared a high proportion of queens (64.7% ± 10, $n = 4$). Because this rate did not differ from

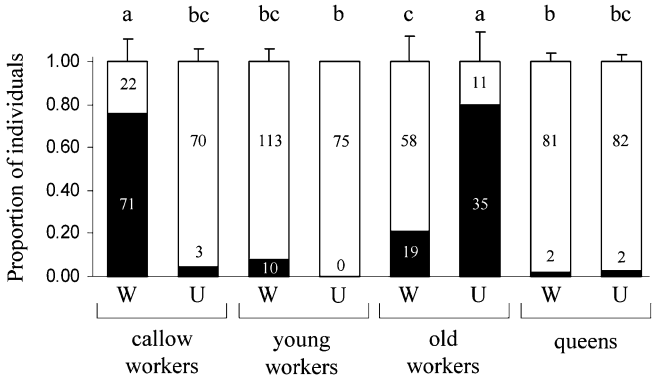

Figure 2
Caste differentiation in larvae of *Cerapachys biroi* according to different rearing and feeding conditions. Data for each experimental condition are mean proportions (±SD) of larvae that developed into worker (white) or ergatoid queen (black) in the 4 replicates. Sizes of each caste produced are given in columns. Different letters represent the rearing conditions that differ significantly (factorial ANOVA followed by a post hoc HSD Tukey's test). Feeding conditions: W = Well-fed and U = Undernourished.

well-fed and isolated colonies of callow workers (one-way ANOVA, volatile pheromone effect: $F_{1,6} = 2.508$; $P = 0.164$), this result rules out the involvement of a volatile pheromone and is consistent with the widespread hypothesis of a fertility signaling transmitted by contact (Monnin 2006; Peeters and Liebig 2009).

Another point is that although cannibalism regularly occurred in colonies and was significantly higher (one-way ANOVA, food effect: $F_{1,30} = 13.366$; $P = 0.00097$) in undernourished conditions (mean ± SD: 39.25% ± 19.8, $n = 16$) compared with well-fed conditions (mean ± SD: 14.69% ± 17.7, $n = 16$), it cannot account for the results obtained: There was no difference in cannibalism according to the type of individuals that reared the larvae (one-way ANOVA, (sub)caste effect: $F_{3,28} = 0.758$; $P = 0.526$), as well as no interaction between feeding conditions and the type of individuals that reared the larvae (factorial ANOVA: $F_{3,24} = 2.417$; $P = 0.091$).

Moreover, queen production was never significantly correlated with cannibalism within colonies of *C. biroi* (Pearson's correlation test, $n = 32$, $R = 0.033$, $P = 0.853$). This turned out to be true in undernourished conditions ($n = 16$, $R = 0.172$, $P = 0.530$), well-fed conditions ($n = 16$, $R = -0.006$, $P = 0.980$), fertile conditions ($n = 20$, $R = -0.294$, $P = 0.209$), and in sterile conditions ($n = 12$, $R = -0.017$, $P = 0.954$). Behavioral observations during the experiments also revealed that adults do not starve larvae and that the latter never present scars.

DISCUSSION

Our study clearly demonstrates that only a low proportion of larvae differentiate into queens under the influence of fecund individuals (fertile workers and queens). These low rates of queens obtained under fertile conditions were similar to the ones observed in field colonies (Ravary F, personal communication) and in colonies reared in the laboratory (3.7–6.3%, Ravary and Jaisson 2004). Moreover, the results show that there was no food effect on larval differentiation and that queen production was not correlated with cannibalism whatever the experimental conditions. This indicates that, despite their high mobility and active feeding behavior and even in the absence of a fertility signal, larvae of *C. biroi* do not seem to selfishly influence their caste development toward queens by securing more food.

Our results can therefore be explained according to our main hypothesis that the fertility of the nursing individuals modulates the queen production. Because fertile individuals reared low proportion of queens in opposite feeding conditions, we suggest that, whatever the food availability, fecund individuals (mainly young workers and queens) could produce a fertility signal that induces a worker differentiation in the developing larvae they care for.

However, because there is an interaction between the type of rearing individuals and the food resources, we also suggest that when this signal is weak (callow and old workers), queen production could be affected by food availability because it may lead to differences in the pheromone regime experienced by larvae. Indeed, well-fed conditions allow larvae to pupate in a few days (mean ± SD: 12.6 days ± 1.7, $n = 16$). Therefore, most well-fed larvae reared by callow workers developed into queens because they pupate before workers are able to lay eggs (i.e., before workers produce the fertility signal). On the contrary, undernourished conditions lead to an increase (24.5 days ± 6.8, $n = 16$) of the typical duration of the larval stage. As 20 days old workers may produce the fertility signal because they usually lay eggs during their first statary phase (Lecoutey E, personal observation), we hypothesize that undernourished larvae initially reared by callow workers were submitted to the fertility signal during their last larval instar and thus developed into workers. Consequently, we predict that if callow workers were continually replaced by younger ones every few days, a high proportion of larvae would develop into queens even if undernourished.

Furthermore, whatever the amount of food received, the production of the fertility signal may decrease in old workers due to their ovarian/physiological senescence, which could explain the increased production of queens. This is reinforced by the fact that only few eggs were laid during the statary phase in each experimental colony of old workers. It has also been reported that, with age, a behavioral shift occurs in old workers, which then hardly ever exhibit brood care in the presence of younger workers (Ravary and Jaisson 2004). Therefore, as foraging activity is regulated by brood satiety in insect societies (Pankiw 2004; Ravary et al. 2006; Pankiw 2007; Mas and Kölliker 2008) and mainly performed by old workers in *C. biroi* (Ravary and Jaisson 2004), the caste ratio difference observed between the 2 feeding conditions in colonies of old workers could be interpreted according to the amount of brood stimulation: In starving conditions, high brood stimulation may have led most of the old workers to neglect nursing activities for foraging (i.e., nest exploration in our experiment because the nests were closed to limit differences in feeding and nursing levels between experimental conditions), whereas in well-fed conditions, old workers may have provided more care to satiated larvae and hence more fertility signal. This latter hypothesis leads to the testable prediction of a positive correlation between "brood contact by workers" and food availability in colonies of old workers.

In the light of all these results, we therefore suggest that worker differentiation is probably induced by fecund individuals via a nonvolatile signal of fertility to which larvae are exposed during brood care. The action of this possible contact pheromone is likely to be dose-dependent and not an all-or-nothing mechanism: The less fertility signal a larva receives, the more pronounced the queen differentiation will be. This hypothesis is supported by 2 observations: First, queens exhibit a well coordinated and graded development of morphological characteristics ranging from different ovariole numbers to variable degrees of fusion of the thoracic tergites. Second, the most developed queens (deep thoracic sutures, vestigial eyes, and 6 ovarioles) were mostly produced in sterile conditions (Lecoutey E, personal observation).

In most social insect species, allocating egg laying to 1 or a few reproductives has some obvious advantages (Oster and Wilson 1978; Hölldobler and Wilson 1990). However, every rose has its thorn: The queens' death usually leads the colony to dwindle to its death (but see Peeters and Molet 2010). In contrast, as thelytokous reproduction is distributed among all nestmates (Ravary and Jaisson 2004), colonies of *C. biroi* are virtually everlasting. In natural colonies of *C. biroi* composed of queens and workers of different ages, brood care is performed by young individuals (callow and fertile individuals) and foraging activities by old workers (Ravary and Jaisson 2004). Consequently, whatever the food resources, fertile colonies may produce only a few queens thus promoting colonial productivity by compelling most larvae to develop into workers. However, workers of *C. biroi* represent the main reproductive force of the colony (94% of workers laying 1–2 eggs during 3–4 months on average vs. 6% of ergatoid queens laying 5 eggs/month on average; Ravary and Jaisson 2004) and only reproduce in their youth (1 or 2 eggs each month during 3–4 months on average, Ravary and Jaisson 2004). Specialized in myrmicine brood predation, colonies of *C. biroi* may sometimes have to face long periods of food shortage resulting in a weak generation turnover. In that

case, colonies are then mainly composed of old workers with decreasing fertility. This may allow many larvae to differentiate into queens especially when food resources are low because a high brood stimulation increases foraging, thus reducing nursing activities. Because queens lay between 4 and 8 eggs during each statary phase (Ravary and Jaisson 2004), producing them instead of workers in a undernourished and senescent society of *C. biroi* could boost the reproductive capacity of the colony by a fourth fold in a sole reproductive cycle.

In senescent societies, such a mechanism also equips the colony with lastingly fecund individuals (laying activities for at least 1 year; Lecoutey E, personal observation) who keep the society fertile even through long periods of starvation. In this obligatory-dependent colony founding species, the production of many queens may also promote budding toward more favorable ecological niches.

In addition, a high proportion of queens is also obtained when larvae are reared by callow workers. Although rarer, this situation can occur when highly fertile colonies experience 2 consecutive cycles of foraging bonanza. In that case and due to the centrifugal polyethism of social insects, well-fed larvae are mainly reared by many callow workers (originating from the previous reproductive cycle) that do not become fertile before they pupate. In these flourishing conditions, the production of numerous queens in *C. biroi* could be a prerequisite to colonial dispersion through budding events during the following cycles.

We therefore propose that a self-regulated mechanism of caste differentiation emerges from the uncommon reproductive division of labor of *C. biroi* in which young workers with finite fertility care for the brood and senescent ones forage. This could be an adaptive mean for *C. biroi* colonies to modulate their caste ratio in response to their level of fecundity.

The genus *Cerapachys* is composed of more than 240 essentially monogynous species (Brown 1975, www.antweb.org), among whom some possess permanently wingless queens (i.e., ergatoid queens). It seems likely that *C. biroi* evolved from an ancestor that had ergatoid queens that mated. As a result of thelytokous parthenogenesis evolving in queens and workers, the queens lost their reproductive monopoly. It is likely that some ecological constraints (i.e., finding a mate) have then favored thelytoky instead of mating. In such a species where all individuals reproduce, the production of thelytokous queens thus became optional and these latter are, at present, mostly produced under the extreme conditions emphasized in our study.

It seems plausible that selection at the colony level may favor the production of queens under extreme conditions (food shortage and senescent colonies or ad libitum food resources and highly fertile colonies), whereas promoting colony growth through worker production when these extreme situations are absent. Interestingly, queen differentiation could also be driven by an individual selection for selfish reproduction. This occurs in *Pristomyrmex punctatus*, another parthenogenetic ant in which it has been recently shown that queens can form a cheater lineage isolated from workers (Dobata et al. 2009). Such parasitic traits are expected to evolve in genetically heterogeneous colonies. However, these possibilities remain to be investigated in *C. biroi*.

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